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14. ABSTRACT

The projects in this proposal specifically target several signal transduction pathways known to be critical for NSCLC p athogenesis including the EGFR pathway and the more downstream ras/raf/Mek/ERK pathway. These projects combine targeted approaches using molecular and imaging techniques to validate activity against a target and monitor response using imaging modalities specific to the receptor using either small molecules or targeted peptide approaches.

15. SUBJECT TERMS
Lung cancer, molecular markers, molecular imaging, targeted therapy

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INTRODUCTION

Lung cancer is the most prevalent cancer worldwide and the leading cause of cancer-related mortality in both men and women in the United States. Conventional multimodality therapies (surgery, radiation and chemotherapy) have reached a therapeutic ceiling in improving the five-year overall survival rate of non-small cell lung cancer (NSCLC) patients, clinically in large part due to chemo- and radiation-resistant locoregional and metastatic spread but ultimately due to poor understanding of the disease and its resistance to the therapy.

Lung cancer is a heterogeneous disease, resulting from accumulated genetic abnormalities over years, which thus requires a coordinated attack in a truly integrated fashion on multiple altered signal pathways. Emerging targeted therapy aims to target key molecular abnormalities in cancer and has succeeded in some tumor types such as chronic myeloid leukemia (CML) (Druker et al., 2004; Druker and Sawyers et al., 2001; Druker and Talpaz et al., 2001), gastrointestinal stromal tumor (Demetri et al., 2002), colon cancer (Hurwitz et al., 2003), and breast cancer (Howell et al., 2005). Thus, the incorporation of targeted therapy into conventional treatments appears to be a new promising approach to treatment of lung cancer.

The program project IMPACT has proposed to integrate targeted therapy in the lung cancer research program when initial clinical results showed disappointing response rates and survival benefit of epidermal growth factor receptor (EGFR) inhibitor gefitinib (Iressa™) for non-selected lung cancer patients (Herbst et al., 2002, 2003, 2004; Herbst, 2004; Kris et al., 2003; Giaccone et al., 2004). It aims to validate molecular mechanisms of targeted agents alone and in combination with chemo- and/or radiation therapies in preclinical and clinical settings. It also aims to develop effective molecular imaging and cancer cell-targeted peptide-based delivery tools to help improve efficacy of the targeted agents. Specifically, our objectives are:

- To validate preclinically and clinically several key signaling pathways and their agents for therapeutic potentials alone or in combination with each other or with chemo and /or radiotherapy
- To explore applications of molecular imaging for targeted therapy and identify cancer cell-targeted peptides for systemic delivery of therapeutic and imaging agents
- To discover and evaluate new molecular abnormalities and therapeutic predictors in lung cancer
- To develop an educational program for teens and young adults for smoking risk and resultant lung cancer occurrence.

IMPACT is composed of 6 research projects, 1 Biostatistics Core, 1 Molecular Pathology Core, 1 Molecular Imaging Core, 2 career development projects, and 2 developmental research projects. Here we present their scientific progresses in the sixth grant year as follows. We note that an additional no-cost extension for this grant has been requested, which is pending review, to allow completion of the clinical activities proposed in this report.

Project 1: Targeting epidermal growth factor receptor signaling to enhance response of lung cancer to therapeutic radiation.

(PI and co-PI: Raymond E. Meyn, Ph.D., Ritsuko Komaki, M.D.)

In spite of significant technical advances including intensity-modulated radiation therapy (IMRT) and chemoradiation, locally advanced lung cancer continues to have a dismal prognosis as many patients' tumors appear to be resistant to radiation therapy. The molecular basis for radiation resistance is not fully understood, but tumor cells have an enhanced survival response that involves increased capacity for DNA repair and suppressed apoptosis. Both apoptosis propensity and DNA repair capacity are thought to be partly controlled by the upstream signal transduction pathways triggered by EGFR activation, which is constitutively activated in many NSCLCs, and its activation leads to a radiation-resistant phenotype. We hypothesize that the response of NSCLC to radiation can be improved through the use of inhibitors of EGFR signaling.

Aim 1 To test the combination of external beam radiation and the selective EGFR-tyrosine kinase inhibitor erlotinib (Tarceva) in locally advanced NSCLC.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 2 To test the hypothesis that activation of the EGFR pathway leads to radiation resistance in NSCLC cells due to an enhanced capacity for repairing DNA lesions.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 3 To test the hypothesis that clinically useful inhibitors of EGFR signaling abrogate DNA repair capacity, restore apoptotic response and radiosensitize NSCLC cells.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 4 To test the hypothesis that targeting both EGFR and its downstream signaling pathways will have at least an additive radiosensitizing effect on NSCLC.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 5 To test whether the strategies developed in Specific Aims 2-4 have efficacy in a xenograft tumor model.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Project 2: Molecular Imaging of EGFR Expression and Activity in Targeting Therapy of Lung Cancer

(PI and co-PI: Juri Gelovani, M.D., Ph.D.; Roy Herbst, M.D., Ph.D.)

Aim 1 To synthesize novel pharmacokinetically optimized ^{124}I and ^{18}F-labeled IPQA derivatives for PET imaging of EGFR kinase activity and conduct *in vitro* radiotracer accumulation studies in tumor cells expressing different levels of EGFR activity.

Summary of Research Findings
This aim was completed and summarized in the previous reports.

Aim 2 To assess the biodistribution (PK/PD) and tumor targeting by novel ^{124}I and ^{18}F-labeled EGFR kinase-specific IPQA derivatives using PET imaging in orthotopic mouse models of lung cancer and compare *in vivo* radiotracer uptake/retention with phospho-EGFR levels *in situ*.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 3 Using selected ^{124}I or ^{18}F-labeled IPQA derivative, to conduct pre-clinical studies in animals with orthotopic models of lung cancer xenografts with different levels of EGFR expression/activity, and to assess the value of PET imaging as the inclusion criterion for therapy by EGFR inhibitors, as well as for monitoring the efficacy of treatment with EGFR-targeted drugs.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 4 Perform pilot clinical PET imaging studies with the optimized ^{124}I or ^{18}F-labeled IPQA derivative under the RDRC guidelines in patients with NSCLC undergoing adjuvant therapy before tumor resection or biopsy. Compare PET image-based measures of EGFR activity with immunohistochemical measures of phospho-EGFR *in situ*.

Summary of Research Findings

The complete IND application for ^{18}F-PEG6-IPQA (IND#111976) was submitted to FDA on April 11, 2011, under section 505(i) of the Federal Food, Drug, and Cosmetic Act and was approved as of May 12, 2011. The trial was activated as of February 27, 2012; however, finalization of the contract between the manufacturer of product, Cyclotope, and the Department of Defense held up active screening for the trial until the contract was executed on May 16, 2012, and the protocol was formally presented to the thoracic faculty and research staff in June 2012. Coordination between the thoracic group and the CABIR faculty was finalized and Dr. John Heymach, Thoracic Section Chief, was added as a collaborator. Subsequently, a dry run of the imaging day procedures was held at the Center for Advanced Bio-Medical Imaging Research (CABIR) facility with participating research staff and research technologists, resulting in successful delivery of the radioactive product to the institution. Additionally, the pharmacokinetic laboratory has performed HPLC runs on human serum spiked with ^{18}F-Fluoro-PEG6-IPQA

without difficulty. The method for the analysis of metabolites in blood and urine samples has been established, together with calibration curves and intercalibration with the gamma counter. Our PK scientist has maintained ongoing routine calibrations and quality assurance procedure for proper maintenance of the machines. All the instruments (HPLCs and gamma counter) are currently functioning properly and ready to be used for PK analysis. Currently, Cyclotope has consistently met the drug release parameters (QC criteria) necessary for the manufacturing and release of the 18F-Fluoro-PEG6-IPQA.

Although the technical capabilities to support the protocol have been in place, we have encountered extreme difficulties in accruing patients to this novel trial. More than 2,600 patients have been screened, of which 471 females had a diagnosis of adenocarcinoma, and 92 had positive EGFR mutations documented. One reason for lack of successful accrual is apparently an exclusion criterion that prohibits subjects with prior EGFR tyrosine kinase inhibitor (TKI) or anti-EGFR monoclonal antibody therapy. A second reason for lack of accrual was determined to be concerns with the length of the wash-out periods currently required before and after administration of this agent, which would unduly delay the patient's treatment.

We have requested a no-cost extension to allow us to seek approval to revise the protocol accordingly and complete the study over the next year. Our plan is to submit the following protocol revisions to the DoD, IRB, and FDA to facilitate successful accrual to the trial:

1. **Broadening the eligibility criteria to include patients previously treated with EGFR TKIs**. This change is proposed for two reasons: first, an important potential use of this imaging agent is to identify tumors that are unlikely to respond to EGFR tyrosine kinase inhibitors (TKIs) because of secondary alterations, such as the T790M resistance mutations, that prevent binding of the TKI or imaging agent to the receptor. We will be able to address this issue by including patients who have progressed after EGFR TKI therapy in addition to treatment-naive patients. Second, previously treated patients typically have an interim period without treatment between treatment regimens; therefore, a delay in treatment is more easily tolerated in this population, as we observed in the previous D0D-sponsored "BATTLE-1" study (Kim et al, Cancer Discovery 2011).

2. **Reducing washout periods prior to and after administration of the agent.** It has now been established through randomized phase III trials that EGFR TKIs such as erlotinib and gefitinib improve cancer-related symptoms and prolong progression-free and/or overall survival, as compared to standard therapy alone in patients with EGFR mutations Given these established benefits, delaying the administration of erlotinib for a prolonged period in NSCLC patients bearing EGFR mutations is not felt to be in the best interests of the patient. Therefore, based on input from treating physicians, we will propose to reduce the washout periods prior to and after administering IPQA.

Key Research Accomplishments:

- Received FDA approval of the IND and activated the Phase I clinical trial of the novel PET imaging agent, ^{18}F-PEG6-IPQA.
- Demonstrated capabilities to manufacture and deliver IPQA compound, meeting all quality standards.
- Assembled a highly experienced research team in place, and initiated extensive screening efforts for patients potentially eligible for the trial.

Conclusions

We have encountered extreme difficulties in accruing patients to the clinical phase I trial studying the novel imaging agent ^{18}F-PEG6-IPQA. Reasons for the lack of successful accrual include: 1) an exclusion criterion that prohibits subjects with prior EGFR tyrosine kinase inhibitor (TKI) or anti-EGFR monoclonal antibody therapy, and 2) concerns with the length of the wash-out periods currently required before and after administration of this agent, which would unduly delay the patient's treatment. We have requested a no-cost extension to allow us to seek approval to revise the protocol accordingly and complete the study over the next year. If this request is granted, our plan is to submit the appropriate protocol revisions to the DoD, IRB, and FDA to facilitate successful accrual to the trial. The results of this effort will be reported in the next annual report.

Reportable Outcomes:

None to date.

Project 3: Targeted Peptide-based Systemic Delivery of Therapeutic and Imaging Agents to Lung Cancer

(PI and co-PI: Renata Pasqualini, Ph.D., Wadih Arap, M.D., Ph.D.)

The studies outlined in this proposal focus on the use of peptide sequences with selective lung tumor-targeting properties. We will seek to validate these probes as delivery vehicles in drug and gene-targeting approaches. This approach directly selects *in vivo* for circulating probes capable of preferential homing into tumors. The strategy will be to combine homing peptides in the context of phage as gene therapy vectors. Given that many of our peptides also target angiogenic vasculature in addition to tumor cells, these studies are likely to enhance the effectiveness of therapeutic apoptosis induction and imaging technology.

Aim 1 To select peptides targeting primary and metastatic tumors in lung cancer patients.

Summary of Research Findings

This aim was completed as reported in the previous annual report.

Aim 2 To validate receptors for targeting human lung cancer.

Summary of Research Findings

This aim was completed as reported in the previous annual report.

Aim 3 To design tools for molecular imaging of lung tumors.

Summary of Research Findings

This aim was completed as reported in the previous annual report.

Project 4: Inhibition of bFGF Signaling for Lung Cancer Therapy

(PI: Reuben Lotan, Ph.D.)

The survival of lung cancer patients is poor because this cancer is diagnosed at advanced stages. Therefore, improvements in early detection through the identification of molecular markers for diagnosis and for intervention combined with targeted chemoprevention are urgently needed. While the molecular events involved in lung cancer pathogenesis are being unraveled by ongoing large scale genomics, proteomics, and metabolomics studies, it is already well recognized that proliferation-, survival- and angiogenesis- promoting signaling pathways are amplified in lung cancer. Among the angiogenesis signaling pathways, the basic fibroblast growth factor (bFGF) and its transmembrane tyrosine kinase receptors (FGFRs) are playing important roles in addition to the well-studied vascular endothelial growth factor (VEGF) and its receptors (VEGFRs). Both types of angiogenesis signaling pathways, the VEGF/VEGFR and the bFGF/FGFR, have been detected in NSCLC and associated with lung cancer development. However, most efforts in preclinical and clinical trials have been directed to the VEGF/VEGFR pathway.

We hypothesize that bFGF triggers signaling pathways that contribute to malignant progression of lung cancers by stimulating tumor cell and endothelial cell proliferation and survival and augmenting angiogenesis. Therefore, agents that intervene in this pathway may be useful for lung cancer therapy either alone or in combination with agents that target the VEGF/VEGFR signaling pathways and/or with cytotoxic agents. We will address the following specific aims in order to understand the mechanism(s) underlying the *in vitro* and *in vivo* effects of bFGF on lung cancer and endothelial cells and the ability of bFGF inhibitors to suppress the growth of NSCLC *in vitro* and *in vivo.*

Aim 1 Determine the effects of bFGF on *in vitro* growth, survival, motility, invasion and angiogenesis of NSCLC cells and endothelial cells.

Summary of Research Findings
This aim was completed and summarized in the previous reports.

Aim 2 Evaluate the relative potency of several inhibitors of bFGF binding to receptor (i.e., TMPP and analogs) in inhibiting effects of bFGF detected in Specific Aim 1 and evaluate the effects of these inhibitors in combination with paclitaxel on *in vitro* growth and survival of tumor cells.

Summary of Research Findings
This aim was completed and summarized in the previous reports.

Aim 3 Evaluate anti-tumor activity (growth inhibition, apoptosis, suppression of angiogenesis) of the most effective inhibitor identified in Specific Aim 2 when used alone and in combination with paclitaxel in an orthotopic lung cancer model using luciferase-expressing NSCLC cells for *in vivo* bioluminescence imaging of tumor growth and response to treatment.

Summary of Research Findings
This specific aim was abandoned as previously reported.

Aim 4 To investigate the expression of bFGF signaling components (bFGF, FGFR-1, FGFR-2, heparan sulfate, syndecan-1, and FGFR-3) by IHC staining of tissue microarrays (TMAs), and correlate the expression of bFGF/bFGFRs between tumor and non-malignant epithelial cells with angiogenesis.

Summary of Research Findings
This aim was completed and summarized in the previous reports.

Project 5: Targeting mTOR and Ras signaling pathways for lung cancer therapy

(Project Co-leaders: Shi-Yong Sun, Ph.D., Suresh Ramalingam, M.D.)

Aim 1 To determine whether an mTOR inhibitor inhibits the growth of human NSCLC cells via G1 growth arrest or induction of apoptosis, and to identify the molecular determinants of mTOR inhibitor sensitivity.

Summary of Research Findings
This aim was completed and summarized in the previous reports.

Aim 2 To determine whether the effect of mTOR inhibitors on the growth of human NSCLC cells is enhanced in the presence of a PI3K inhibitor or a MAPK inhibitor.

Summary of Research Findings
This aim was completed and summarized in the previous annual report.

Aim 3 To evaluate the efficacies of the combinations of rapamycin with LY294002 or U0126 in nude mice models of lung cancer xenografts *in vivo*.

Summary of Research Findings
This aim was completed and summarized in the previous reports.

Aim 4 To conduct a pilot clinical biochemical induction trial to investigate the effect of RAD001 in operable NSCLC patients and identify molecular determinants of RAD001 sensitivity and prognosis.

Summary of Research Findings
We conducted this 'window- of-opportunity' study to characterize the biologic activity of everolimus, an allosteric inhibitor of mTOR pathway, in patients with surgically resectable NSCLC. Patients with Stage I-III NSCLC underwent baseline tumor biopsy and FDG PET/CT scan followed by treatment with everolimus 5mg daily (Arm A) or 10mg daily (Arm B) for up to 28 days. A repeat PET/CT scan was obtained 24 hours prior to surgery. Blood samples for pharmacokinetic (PK) assay for drug levels were collected at 0.5, 1, 2, 5, 8 and 24 hours post-drug ingestion on Days 1, 8 and 21. Control patients not treated with everolimus (Arm C) also had paired FDG PET/CT scans prior to surgery. Target modulation by everolimus was assessed in vivo by PET and ex vivo by immunohistochemical detection of total and phosphorylated mTOR, Akt, S6, p70S6, eIF4e and 4EBP1 in pretreatment and post-treatment tissue samples. We enrolled 32 patients (Arm A - 12; Arm B - 11; Arm C - 9); median age: 63 yrs (range 35-77);

gender: 14 males, 18 females; stage: I - 14; II - 12; IIIA - 6; histology: adenocarcinoma - 21; squamous - 7; others - 4. Treatment was tolerated well with mostly grade 1/2 toxicities (hyperglycemia, hypertriglyceridemia, anemia and fatigue) and 31 of 32 patients proceeded with surgery on schedule. Paired PET/CT scan in everolimus-treated patients showed a significant reduction in median SUVmax (-21.74%, -23.23% vs. 8.63%; p=0.027 for Arms A, B and C respectively) and anatomic tumor growth arrest as measured by blinded independent CT scan review (-3.13%, 0% vs. 10%; p=0.039 for Arms A, B and C respectively). Comparison of baseline and resected tumor specimens following 3-4 weeks of treatment with everolimus showed effective pathway modulation with a significant difference in mean baseline and post-treatment pS6 immunoscore from 119 to 59, 257 to 115 vs. 181 to 169 for Arms A, B & C respectively; p=0.01 for comparison by dose of everolimus.

Key Research Accomplishments

1. This completed study demonstrates that modulation of downstream targets of mTOR were accomplished with the 10 mg dose of everolimus, but only to a lesser extent with the 5 mg dose. Based on this finding, the 10 mg dose of everolimus should be considered the biologically optimal dose for solid organ tumors. Our findings have significant implications for further development of mTOR inhibitors in NSCLC.
2. We have now developed a novel combination of everolimus with BKM120, a PI3K inhibitor, that is currently being studied in a phase I clinical trial at our institution. This study is based on promising pre-clinical data that showed synergistic inhibitor effects on the growth of human NSCLC cells, both in vitro and in vivo.
3. The above-mentioned combination approach is included as a full project in our pending NCI application for a P01 award in lung cancer.

Reportable outcomes

Taofeek Kunle Owonikoko, Daniel L. Miller, Seth Force, Gabriel Sica, Scott Arthur Kono, Nabil F. Saba, Madhusmita Behera, Zhengjia Chen, Allan Pickens, Jennifer Mendel, Robert Fu, William F Auffermann, Jaqueline Willemann Rogerio, William E Torres, Haian Fu, John A. Hohneker, Shi-Yong Sun, Anthony A Gal, Suresh S. Ramalingam, Fadlo Raja Khuri. A phase IB window-of-opportunity study of everolimus in patients with resectable non-small cell lung cancer (NSCLC). Submitted as an abstract to the annual ASCO meeting, 2013.

Conclusions

Everolimus exerts a measurable, dose-dependent biologic activity in NSCLC tumors. 'Window of opportunity' studies in early stage NSCLC provide strong mechanistic insights and may guide development of novel targeted agents.

Project 6: Identification and Evaluation of Molecular Markers in Non-Small Cell Lung Cancer (NSCLC)

(PI and co-PI: Ralf Krahe, Ph.D., Li Mao, M.D)

A better understanding of the lung cancer biology and an identification of genes involved in tumor initiation, progression and metastasis are an important first step leading to the development of new prognostic markers and targets for therapy. In the same context, identification of reliable predictive markers for response or resistance to therapy in NSCLC patients is also desperately desired for optimal delivery of targeted therapy and/or standard

chemotherapy. The proposed studies aim to identify the two types of markers that would eventually help develop smarter clinical trials, which will selectively recruit patients who are more likely to respond to one regimen over another and lead to improvement of overall therapeutic outcomes.

Aim 1 To expression profile by DNA microarray technology aerodigestive cancers - with primary focus on adenocarcinoma and squamous cell carcinoma (SCC) of the lung, and head and neck squamous cell carcinoma (HNSCC), including primary tumors and normal adjacent tissue, and (where available) metastatic lesions.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 2 To DNA profile the same samples by complementing DNA approaches to stratify RNA expression profiles on the basis of their corresponding DNA profiles.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 3 To evaluate the contribution of promoter hypermethylation and transcriptional inactivation of known cancer genes subject to epigenetic silencing to cancer phenotype.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 4 To determine protein signatures of treatments of erlotinib and other therapeutic agents, alone or in combination, in NSCLC and identify molecular predictors of response.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 5 To determine a clinical utility of the molecular predictors.

Summary of Research Findings
This aim was completed and summarized in previous reports.

Core B: Biostatistics & Data Management Core

(Core Director: J. Jack Lee, Ph.D.)

The Biostatistics and Data Management Core has continued to work with all IMPACT Projects in their research efforts, especially in the area of biostatistical support in clinical trial design, implementation, and analysis of experimental results. We also developed statistical methods to enhance the design and analysis pertinent to the lung cancer research.

Specific Aims:

1. To ensure that the results of all projects are based on well-designed experiments and are appropriately interpreted by providing experimental design; sample size estimates; power calculations; and integrated, comprehensive analysis for each basic science, pre-clinical, and clinical study.
2. To develop a data management system that integrates clinical, pathological, and basic science data while providing data integrity through process tracking and quality control.
3. To provide statistical and data management support for genomic and imaging studies including microarray, proteomics, and molecular targeted imaging.
4. To develop and adapt innovative statistical methods pertinent to biomarker-integrated translational lung cancer studies.
5. To produce statistical reports for all projects.
6. To collaborate and assist all project investigators with the publication of scientific results.

Summary of Research Findings and Key Accomplishments

For Project 2, "Molecular Imaging of EGFR Expression and Activity in Targeting Therapy of Lung Cancer," Core B worked with study investigators in the development and revision of a three-stage design for the protocol "A phase I study of 18F-Fluoro-PEG6-IPQA as a PET Imaging Agent for Active/Mutant EGFR Expression in Tumors (2009-0832)." In addition, Core B personnel continued to develop statistical methods for biomarker-integrated novel clinical trial designs for lung cancer-targeted therapies.

Conclusions

Core B continued to provide statistical support for Project 2, including the development of relevant statistical methodologies.

Core C: Pathology Core

(Director: Ignacio Wistuba, M.D.)

The IMPACT interdisciplinary research proposal for studying targeted therapy of lung cancers has required extensive histopathologic, IHC, and molecular studies of cell and tissues specimens, which have been assisted, coordinated or performed by the Pathology Core. One of the most important roles of the Pathology Core has been to provide professional technical services for proper procurement, storage and use of human and animal tissues, as well as technical assistance for IHC analysis. In addition, the Pathology Core has provided assistance for collection and evaluation of tissue specimens in IMPACT clinical trials in lung cancer patients.

Aim 1 Develop and maintain repository of tissue, cell and serum specimens from patients with lung neoplasia, as requested by the various component projects.

Summary of Research Findings

This aim was completed as reported in the previous annual report.

Aim 2 Develop innovative tissue and cell reagents from lung cancer patients for the investigation and validation of the molecular endpoints relevant to each component project.

Summary of Research Findings

This aim was completed as reported in the previous annual report.

Aim 3 Process human and animal cell and tissues for histopathological, immunohistochemical (IHC) and molecular analyses, including tissue microdissection, as required by each component project.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 4 Perform and evaluate IHC analysis in human and animal cell and tissue specimens, as required by the various component projects.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

DRP-1: Treatment of Malignant Pleural Effusion with ZD6474, a Novel VEGFR and EGFR TK Inhibitor

(PI and co-PI: Roy Herbst, M.D., Ph.D., Carlos Jimenez, M.D.)

Recurrent malignant pleural effusion is a debilitating clinical problem that requires palliation with repeated therapeutic thoracentesis or pleurodesis (Putnam, J Surg Clin North Am 2002). Malignant pleural effusions have been associated with high levels of VEGF. Treatment with a VEGFR tyrosine kinase inhibitor resulted in a decrease in the amount of pleural effusion in an animal model (Yano, CCR 2000). We hypothesize that malignant pleural effusion formation in cancer patients can be decreased with ZD6474 (AstraZeneca), a VEGFR and EGFR tyrosine kinase inhibitor.

Aim 1 To determine clinical effect of ZD6474.

Aim 2 To investigate biological correlates.

Aim 3 To investigate radiographic correlates.

Aim 4 To assess quality of life.

Summary of Research Findings
This study was completed as reported in the previous annual report.

DRP-2: TALK - Teens and Young Adults Acquiring Lung Cancer Knowledge

(PI: Alexander V. Prokhorov, M. D., Ph.D.)

Ninety percent of lung cancer cases in adults are direct results of smoking. In children and young adults, tobacco use remains a major public health problem in spite of the recent declines in smoking prevalence among children and adolescents. Over the past 2-3 decades, numerous factors of smoking initiation among adolescents have been thoroughly investigated. A considerable volume of literature is currently available providing important clues with respect to designing tobacco prevention and cessation among youth.

Focusing on this major public health problem – tobacco use among young individuals and lack of in-depth knowledge of lung cancer issues – Project TALK (Teens and Young Adults Acquiring Lung Cancer Knowledge) was conceived and funded as a smoking cessation/prevention pilot project for culturally diverse high-risk young populations that include school drop-outs, economically disadvantaged, and underserved. Using modern technologies, the Departments of Behavioral Science and Thoracic/Head & Neck Medical Oncology have joined their efforts to conduct this developmental project under the leadership of Dr. Alexander V. Prokhorov. The project will assist in making major advances in lung cancer education and prevention among youth. Project TALK will produce a CD-ROM-based education/behavior change for teenagers and young adults (15-24 years of age).

We have thus been devoting our effort in 4 tasks as described in the Statement of Work based on the project timeline:

Aim 1 **Develop intervention program.** Focus groups will be held with adolescents and young adults to ensure we are capturing the essence of the program, using the right messages, and employing the appealing video and animated characters. (Years 1-2)

Summary of Research Findings
This aim was completed and summarized in previous reports.

Aim 2 **Develop and beta-test CD-ROM.** This includes the design of the animation, illustrations, scripts and accompanying videos. (Years 1-2)

Summary of Research Findings
This aim was completed and summarized in previous reports.

Aim 3 **Implement program in agreed upon locations and recruit young adults to participate in the study.** (Years 3-4)

Summary of Research Findings
This aim was completed and summarized in previous reports.

Aim 4 **Collect and analyze data.** (Years 3-4)

Summary of Research Findings
This aim was completed and summarized in previous reports.

Career Developmental Project (CDP1): Identification of Membrane Proteins in Bronchial Epithelia Cells as Biomarkers of Early Detection for Lung Cancer

(PI: Ja Seok Peter Koo, Ph.D.)

Lung cancer is the leading cause of cancer deaths. Early detection of the malignant lesion leads to an improved 5-year survival rate after surgical resection. Therefore, advanced screening tools are needed urgently to detect lung cancer at an early stage to improve control of such deadly lung cancer.

Aim 1 To isolate membrane proteins uniquely expressed on the surface of squamous metaplasia using organotypically cultured bronchial epithelial cells.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 2 To identify differentially represented proteins using proteomics.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

Aim 3 To verify the differentially represented proteins using PCR, Western blotting, and immunocytochemistry.

Summary of Research Findings
This aim was completed as reported in the previous annual report.

KEY RESEARCH ACCOMPLISHMENTS

Project 2: Molecular imaging of EGFR expression and activity in targeted therapy of lung cancer

1. Received FDA approval of the IND and activated the Phase I clinical trial of the novel PET imaging agent, ^{18}F-PEG6-IPQA.
2. Demonstrated capabilities to manufacture and deliver IPQA compound, meeting all quality standards.
3. Assembled a highly experienced research team in place, and initiated extensive screening efforts for patients potentially eligible for the trial.

Project 5: Targeting mTOR and Ras signaling pathways for lung cancer therapy

1. This completed study demonstrates that modulation of downstream targets of mTOR were accomplished with the 10 mg dose of everolimus, but only to a lesser extent with the 5 mg dose. Based on this finding, the 10 mg dose of everolimus should be considered the biologically optimal dose for solid organ tumors. Our findings have significant implications for further development of mTOR inhibitors in NSCLC.
2. We have now developed a novel combination of everolimus with BKM120, a PI3K inhibitor, that is currently being studied in a phase I clinical trial at our institution. This study is based on promising pre-clinical data that showed synergistic inhibitor effects on the growth of human NSCLC cells, both in vitro and in vivo.
3. The above-mentioned combination approach is included as a full project in our pending NCI application for a P01 award in lung cancer.

Core B: Biostatistics & Data Management Core

For Project 2, "Molecular Imaging of EGFR Expression and Activity in Targeting Therapy of Lung Cancer," Core B worked with study investigators in the development and revision of a three-stage design for the protocol "A phase I study of 18F-Fluoro-PEG6-IPQA as a PET Imaging Agent for Active/Mutant EGFR Expression in Tumors (2009-0832)." In addition, Core B personnel continued to develop statistical methods for biomarker-integrated novel clinical trial designs for lung cancer-targeted therapies.

REPORTABLE OUTCOMES

Publications:

None to date.

Abstracts:

Taofeek Kunle Owonikoko, Daniel L. Miller, Seth Force, Gabriel Sica, Scott Arthur Kono, Nabil F. Saba, Madhusmita Behera, Zhengjia Chen, Allan Pickens, Jennifer Mendel, Robert Fu, William F Auffermann, Jaqueline Willemann Rogerio, William E Torres, Haian Fu, John A. Hohneker, Shi-Yong Sun, Anthony A Gal, Suresh S. Ramalingam, Fadlo Raja Khuri. A phase IB window-of-opportunity study of everolimus in patients with resectable non-small cell lung cancer (NSCLC). Submitted as an abstract to the annual ASCO meeting, 2013.

CONCLUSIONS

Project 2: We have encountered extreme difficulties in accruing patients to the clinical phase I trial studying the novel imaging agent ^{18}F-PEG6-IPQA. Reasons for the lack of successful accrual include: 1) an exclusion criterion that prohibits subjects with prior EGFR tyrosine kinase inhibitor (TKI) or anti-EGFR monoclonal antibody therapy, and 2) concerns with the length of the wash-out periods currently required before and after administration of this agent, which would unduly delay the patient's treatment. We have requested a no-cost extension to allow us to seek approval to revise the protocol accordingly and complete the study over the next year. If this request is granted, our plan is to submit the appropriate protocol revisions to the DoD, IRB, and FDA to facilitate successful accrual to the trial. The results of this effort will be reported in the next annual report.

Project 5: Everolimus exerts a measurable, dose-dependent biologic activity in NSCLC tumors. 'Window of opportunity' studies in early stage NSCLC provide strong mechanistic insights and may guide development of novel targeted agents.

Biostatistics Core: Core B continued to provide statistical support for Project 2, including the development of relevant statistical methodologies.